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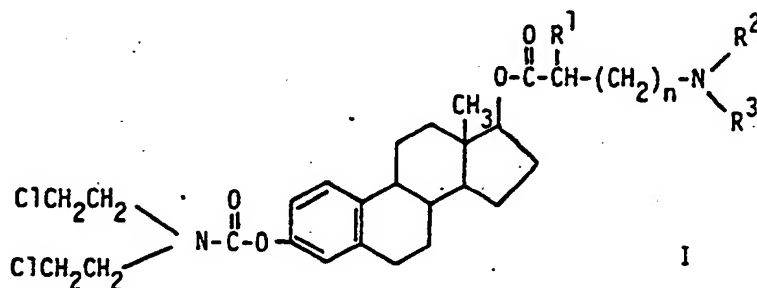
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54 Novel esters.

57 The present invention concerns novel compounds having the general formula



wherein R¹, R² and R³ are selected from the group consisting of hydrogen or lower alkyl having 1-4 carbon atoms, wherein R² and R³ together with the N atom can also form a ring having 2-5 carbon atoms and wherein n is 0, 1 or 2, and pharmaceutically acceptable salts thereof.

The compounds according to the invention are useful as anticancer agents.

NOVEL ESTERS

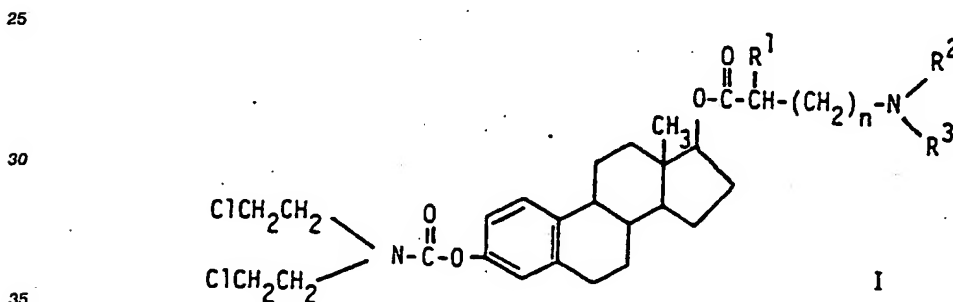
The present invention concerns novel esters having anticancer activity. Specifically the invention concerns novel esters of estramustine.

Estramustine, *estra-1,3,5(10)-triene-3,17 β -diol 3-/N,N-bis-(2-chloroethyl)carbamate/*, and various esters thereof are previously known as antineoplastic agents from e.g. the US patent 3,299,104. One of these esters, 17-estramustine dihydrogen phosphate ester, or abbreviated EMP, has been further developed and water soluble salts thereof, Estracyt^R, are now widely used for treating prostatic cancer. A problem with the water soluble salts of EMP when taken orally concerns the interaction with calcium ions in food and drinks. In the presence of calcium ions these EMP salts give precipitates and form an insoluble complex, which is not absorbed from the gastro-intestinal tract and therefore has very little activity in the body. Several attempts to overcome this problem have been made, both as regards the pharmaceutical preparation of EMP salts and of estramustine as such. It has, however, not been possible to find EMP modifications for oral use, which in the presence of calcium ions produce the same plasma levels of the main EMP metabolites estramustine and the corresponding 17-keto compound, estromustine, as does the water soluble disodium salt of EMP when taken in absence of calcium.

Unexpectedly it has now been found that the problem with the calcium interaction can be avoided and that the plasma levels of estramustine and estromustine as well as the anticancer activity can be maintained at an essentially unchanged or even higher level if the estramustine is administered in the form of certain amino acid esters.

Amino acid esters for pharmaceutical purposes have been described in the British Patent 962,797 and in the European Patent Application 0104746. The aims of the inventions of these applications, however, are different from the aim of the present invention and the alcohols esterified, although containing a steroid skeleton, have structures widely different from that of estramustine.

The present invention concerns novel compounds having the general formula:



wherein R^1 , R^2 and R^3 are selected from hydrogen and lower alkyl having 1-4 carbon atoms, wherein R^2 and R^3 together with the N atom can form a ring having 2-5 carbon atoms, and wherein n is 0, 1 or 2, and pharmaceutically acceptable salts thereof. Preferred compounds are those where $n=0$, R^1 is hydrogen and R^2 and R^3 are equal or different and are hydrogen, methyl or ethyl.

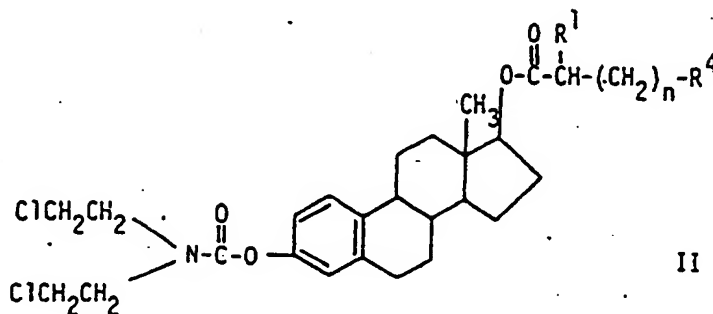
The following compounds are especially preferred.

- 17-estramustine N,N-diethylaminoacetate
- 17-estramustine 4-(N,N-dimethylamino)butyrate
- 17-estramustine N-methylaminoacetate
- 17-estramustine aminoacetate
- 17-estramustine 2-aminopropionate
- 17-estramustine N-ethylaminoacetate
- 17-estramustine N-(2-propyl)aminoacetate
- 17-estramustine 3-aminopropionate
- 17-estramustine N-(1-propyl)aminoacetate

The novel compounds are normally prepared from estramustine using conventional methods, two of them being mentioned below. The preparation of estramustine itself is disclosed in e.g. the US patent 3,299,104.

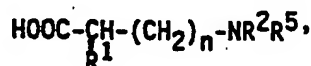
In one of the methods estramustine is esterified with an acid containing a reactive substituent such as

halogen, e.g. chloro, bromo, iodo, or organic sulfonyloxy, organic being a hydrocarbon residue, containing 1 to 6 carbon atoms, giving an intermediate having formula II,



wherein R^4 is the reactive substituent. The intermediate is then reacted with amines HNR^2R^3 giving the novel compounds having formula I.

According to the other method estramustine is esterified with an amino acid having the general formula



wherein R^5 is R^3 or optionally a protecting group, giving an intermediate having the general formula II, wherein R^4 is $-NR^2R^5$, from which the protecting group is afterwards removed for the preparation of compounds having the general formula I. Examples of substituents R^5 protecting the amino group are t-butoxycarbonyl and benzyloxycarbonyl.

For the esterification well known methods are used. One type of method is based on reactions with reactive derivatives of the acids such as acyl chlorides, bromides and mixed anhydrides with organic acids including those obtained from lower alkyl chloroformates. Another type of method is based on reactions with acids in the presence of dehydrating agents, e.g. 1,1-carbonyldiimidazole and N,N'-dicyclohexylcarbodiimide.

The salts of the novel compounds are prepared from the base-form of the compound and pharmaceutically accepted acids, e.g. those listed in International Journal of Pharmaceutics 3, 202 (1986), which is hereby incorporated by reference. Preferred acids are hydrochloric acid, hydrobromic acid, methanesulfonic acid and ethanesulfonic acid.

Among the salts of the novel compounds the following are specially preferred: 17-estramustine N-methylaminoacetate, hydrochloride; 17-estramustine aminoacetate, hydrochloride; 17-estramustine N-methylaminoacetate, methanesulfonate; 17-estramustine aminoacetate, methanesulfonate; 17-estramustine N-methylaminoacetate, ethanesulfonate and 17-estramustine aminoacetate, ethanesulfonate.

Although the compounds according to the invention are at first hand intended for oral use it is obvious that other ways of administration are within the scope of the invention.

Pharmaceutical formulations are thus usually prepared from a predetermined quantity of one or more of the compounds of the invention. Such formulations may take the form of powders, granules (pellets), suppositories, capsules or tablets, suspensions, etc. with or without, but preferably with, any one of a large variety of pharmaceutically acceptable excipients. When in a mixture with a pharmaceutical vehicle or carrier, the active ingredient usually comprises from about 0.01 to 95 percent, normally from about 0.05 to about 80 percent, by weight of the composition. Carriers such as cellulose, sugar, talc, commonly used synthetic and natural gums, natural and synthetic oils, emulsifying and dispersing agents, water, and the like, may be used in such formulations. Binders such as polyvinylpyrrolidone and lubricants such as magnesium stearate, may be used to form tablets. Disintegrating agents such as starch may also be included in tablets.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 to about 1000 mg, more usually about 5 to about 300 mg of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of the new ester calculated to produce the desired

therapeutic effect, in association with the required pharmaceutical carrier.

The compound according to the invention is effective over a wide dosage range. For example, dosages per day will normally fall within the range of about 0.1 to about 100 mg/kg of body weight. In the treatment of adult humans, the range of about 0.5 to about 50 mg/kg, in single or divided doses, is preferred. It will be understood that the amount of the compound actually administered will be determined by a physician; in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. As used herein the terms "pharmaceutical compositions" and "pharmaceutically acceptable" include compositions and ingredients for both human and veterinary use.

The following examples are intended to illustrate but not to limit the scope of the invention, although the compounds named are of particular interest for our intended purposes.

15 Example 1

17-Estramustine chloroacetate

20 Estramustine (4.4 g, 0.01 mol) is dissolved in toluene (100 ml). Chloroacetyl chloride (1.7 g, 0.015 mol) is added and the solution is heated at 70°C for 1.5 hours. The solvent is then removed in a rotary evaporator at 30°C. The residue is treated with ethanol (40 ml) and the product crystallizes. The product is collected on a filter and washed with a few ml of ethanol. The chloroacetate after drying at room temperature melts at 95°C. Yield 4.9 g.

Example 2

30 17-Estramustine bromoacetate

This compound is prepared by a method similar to the one described in example 1 from estramustine and bromoacetyl bromide. M.p. 109°C.

35 Example 3

17-Estramustine N-methylaminoacetate, hydrochloride

40 17-Estramustine chloroacetate (5.2 g, 0.01 mol) is dissolved in acetonitrile (40 ml) at room temperature. Methylamine (3.1 g) (0.1 mol) dissolved in cold acetonitrile (10 ml) at 0°C is added. After 3 h the excess of amine and the solvent is removed in a rotary evaporator. The residue is dissolved in methylene chloride (50 ml) and washed three times with 50 ml water, pH being adjusted each time to 7-9 with a sodium bicarbonate solution. The methylene chloride solution is dried over anhydrous sodium sulfate and the solvent is carefully evaporated. The residue is dissolved in acetonitrile (40 ml) and the N-methylaminoacetate ester is precipitated by the addition of a solution of hydrogen chloride in ether (0.012 mol). A voluminous precipitate is formed which after stirring for a few minutes disintegrates to fine crystals. The product is collected on a filter and washed with a mixture of ethylacetate and acetonitrile (1:1). The hydrochloride is then dried under vacuum at room temperature for 24 h. Yield 3.7 g. M.p. 226°C, with decomposition. NMR spectroscopy shows that the structure of the product is estramustine 17-N-methylaminoacetate.

55 Example 4

17-Estramustine N-methylaminoacetate, methanesulfonate and ethanesulfonate

Using the method described in example 3 except for using a solution of methanesulfonic acid or ethanesulfonic acid in ether instead of hydrogen chloride in ether a methanesulfonate, m.p. 212°C, or an ethanesulfonate, m.p. 170°C, respectively, are obtained (compounds Nos. 4:1 and 4:2, respectively).

Example 5

17-Estramustine N-R²-N-R³aminoacetate, hydrochloride

Esters having the amino substituents R² and R³ described in table 1 are made from 17-estramustine bromoacetate using the method of example 3 with minor modifications.

Table 1

R ²	R ³	Hydrochloride m.p. °C	Amine used
-CH ₃	-CH ₃	210	(CH ₃) ₂ NH
H	-CH ₂ CH ₃	220	CH ₃ CH ₂ NH ₂
H	-CH ₂ CH ₂ CH ₃	170	CH ₃ CH ₂ CH ₂ NH ₂
H	-CH(CH ₃) ₂	200	(CH ₃) ₂ CHNH ₂
H	-C(CH ₃) ₃	172	(CH ₃) ₃ CNH ₂
-CH ₂ CH ₃	-CH ₂ CH ₃	158	(CH ₃ CH ₂) ₂ NH
-CH ₂ -CH ₂ -CH ₂ -CH ₂ -		210	NH

Example 6

17-Estramustine aminoacetate, hydrochloride and methanesulfonate

Estramustine (4.4 g, 0.01 mol) and N-tert-butoxycarbonylglycine (1.75 g, 0.01 mol) are dissolved in methylene chloride (35 ml). Dicyclohexylcarbodiimide (2.1 g, 0.01 mol) and 4-dimethylaminopyridine (0.1 g, 0.001 mol) are added and the reaction mixture is stirred for 3 hours. The dicyclohexylurea formed is removed by filtration and the solvent is carefully evaporated. The oily residue is dissolved in acetonitrile (10 ml). Acetonitrile (40 ml) containing hydrogen chloride (0.03 mol) is added and the mixture is stirred for 16 hours. The precipitate formed is collected on a filter and recrystallized from methanol/ether. Yield 3.2 g of 17-estramustine aminoacetate, hydrochloride, (m.p. 220°C, compound No. 6:1) as confirmed by NMR-spectroscopy.

With the same method except for using methanesulfonic acid (0.03 mol) instead of hydrogen chloride and omitting the recrystallization 17-estramustine aminoacetate, methanesulfonate (compound No. 6:2) is obtained, m.p. 206°C.

Example 7

17-Estramustine aminopropionates, hydrochlorides

Using the method described in example 6 the following salts are obtained:

- 17-estramustine 2-L-aminopropionate, hydrochloride, m.p. 248°C, from N-tert-butoxycarbonyl-L-alanine
- 17-estramustine 3-aminopropionate, hydrochloride, m.p. 223°C, from N-tert-butoxycarbonyl-β-alanine

Example 817-Estramustine aminoacetate, methanesulfonate

- 5 To estramustine (4.4 g, 0.01 mol) dissolved in methylene chloride (30 ml) is added N-benzyloxycarbonylglycine (2.2 g, 0.01 mol), dicyclohexylcarbodiimide (2.25 g, 0.011 mol) and 4-dimethylamino-pyridine (0.12 g, 0.001 mol). The mixture is stirred at room temperature for 2 h. The dicyclohexylurea formed is removed by filtration. The filtrate is washed with hydrochloric acid (2 mol/l, 10 ml), water (10 ml), sodium carbonate solution (1 mol/l, 25 ml) and with water (25 ml) and dried with sodium sulfate. The solvent is evaporated in a rotary evaporator. The residual oil is dissolved in ethanol (125 ml).
 10 Palladium on carbon (1 g, Pd-content 5%) is added and the mixture is treated with hydrogen at atmospheric pressure and room temperature. When the reaction has ceased the mixture is filtered and the solvent is evaporated carefully. The residue is dissolved in acetonitrile (75 ml). Methanesulfonic acid (1 g, 0.01 mol) is added. After stirring of the mixture for 18 h the precipitate is collected on a filter and washed with acetonitrile and dried at room temperature. Yield 3.7 g of 17-estramustine aminoacetate, methanesulfonate, m.p. 204°C.

Example 917-Estramustine 4-(N,N-dimethylamino)butyrate, hydrochloride

- 25 To estramustine (4.4 g, 0.01 mol) in methylene chloride (50 ml) is added 4-dimethylaminobutyl chloride, hydrochloride, (1.3 g, 0.01 mol). The solution is refluxed for 2 h. The solvent is evaporated and the resulting oil crystallizes. The product is treated with activated carbon in ethanol solution and recrystallized from ethanol/ether. Yield 6.5 g. M.p. 205°C. The product is 17-estramustine 4-(N,N-di-methylamino)butyrate, hydrochloride, as confirmed by NMR-spectroscopy.

Example 10Interactions with calcium ions

- Solution A: 0.3 mole/l calcium diethanesulfonate. Calcium carbonate (1.38 g, 15 mmole) is dissolved by adding a 1.0 mole/l ethanesulfonic acid solution until a stable pH=5.3 is obtained. The solution is diluted with water to 50 ml.
 40 Solution B: 0.03 mole/l calcium diethanesulfonate. One part of solution A is diluted with 9 parts of water.
 Solution C: 0.05 mole/l sodium acetate/acetic acid buffer with pH=4.5. Glacial acetic acid (3.0 g, 0.05 mole) is dissolved in 800 ml of water, titrated with 1 mole/l sodium hydroxide solution to pH=4.5 and diluted to 1 l.
 The test compound is dissolved in 50 ml of solution C. A clear solution is obtained in a few minutes.
 45 After 5 minutes an equimolar amount of calcium ions are added by adding 1 ml of solution A or B. The mixture is observed for 3 h.

5	TEST COMPOUND	Amount		Solution added, mmole calcium ions	Observation
		mg	mmole		
	Estramustine phosphate, disodium salt	16	0.03	B, 0.03	Becomes unclear immediately
10	Compound No. 6:2	178	0.3	A, 0.3	Remains clear
	" " 4:2	186	0.3	A, 0.3	" "

15 The results show that the novel compounds in the presence of calcium ions do not give any precipitate at concentrations 10 times higher than the concentrations of estramustine phosphate disodium salt and calcium ions required to give an immediate formation of an undissolved estramustine phosphate calcium salt.

20 Example 11

25 Comparison of estramustine phosphate and novel estramustine esters with respect to oral bioavailability of estramustine in dogs

30 Different formulations were administered orally to groups of four Beagle dogs. The dose was equivalent to 140 mg of estramustine phosphate. The animals were fasting before dosing and given 50 ml 0.01 M hydrochloric acid after the dose. Blood samples were analysed for estramustine by means of gas chromatography. (Andersson, S-B, et al., Acta Pharm.Suec. 19, 1 (1982).

The following formulations were used:

1. Water solution of estramustine phosphate disodium salt
2. Water solution of compound No. 6:2
3. Water solution of compound No. 4:2

35 Table 1 shows the areas under the plasma concentration versus time curves, AUC, of estramustine in dogs. The mean values were 54 ± 62, 118 ± 60 and 83 ± 62 ng/mlxhours for estramustine phosphate, compound No. 6:2 and No. 4:2, respectively.

TABLE 1

Oral bioavailability of estramustine in dogs. AUC-values after administration of solutions of the estramustine esters			
Dog No.	Estramustine phosphate disodium salt	Compound No. 6:2	Compound No. 4:2
8727	147	100	-
8729	16.4	140	94.0
8731	34.0	45.8	79.8
8733	19.5	184.8	-
8735	-	-	2.6
8742	-	-	153.4
Mean±S.D.	54.3 ± 62.4	118 ± 59	82.6 ± 62.0

55 Example 12

Formulation, plain tablets		
I	Compound No. 6:2, mg	160
	Corn starch, mg	15
II	Polyvidone, mg	8
	Ethanol	q.s.
III	Corn starch, mg	15
	Magnesium stearate, mg	2

I is mixed and granulated with solution II. After drying and milling through 1 mm sieve III is added. The mixture is compressed to tablets with a weight of 200 mg.

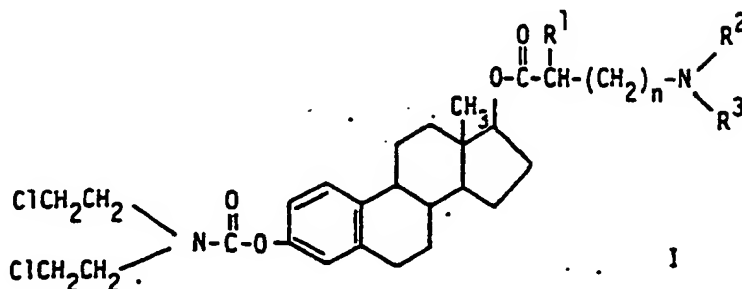
Example 13

Formulation, capsules

The mixture from example 12 is filled into hard capsules, size No. 1.

Claims

1. Novel compounds having the general formula



wherein R^1 , R^2 and R^3 are selected from the group consisting of hydrogen or lower alkyl having 1-4 carbon atoms, wherein R^2 and R^3 together with the N atom can also form a ring having 2-5 carbon atoms and wherein n is 0, 1 or 2, and pharmaceutically acceptable salts thereof.

2. Compounds according to claim 1 characterized in that n is 0.

3. Compounds according to claim 1 or 2 characterized in that R^1 is hydrogen.

4. Compounds according to any of the claims 1-3, characterized in that R^2 and R^3 are equal or different and consist of hydrogen, methyl or ethyl.

5. A compound according to claim 1 selected from the following group:

- 17-estramustine N,N-diethylaminoacetate
- 17-estramustine 4-(N,N-dimethylamino)butyrate
- 17-estramustine N-methylaminoacetate
- 17-estramustine aminoacetate
- 17-estramustine 2-aminopropionate
- 17-estramustine N-ethylaminoacetate
- 17-estramustine N-(2-propyl)aminoacetate
- 17-estramustine 3-aminopropionate
- 17-estramustine N-(1-propyl)aminoacetate

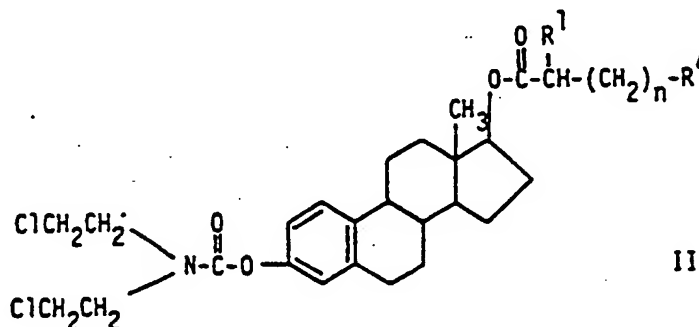
6. A compound according to claim 1 selected from the following group:

- 17-estramustine N-methylaminoacetate, hydrochloride

17-estramustine aminoacetate, hydrochloride
 17-estramustine N-methylaminoacetate, methanesulfonate
 17-estramustine aminoacetate, methanesulfonate
 17-estramustine N-methylaminoacetate, ethanesulfonate
 5 17-estramustine aminoacetate, ethanesulfonate

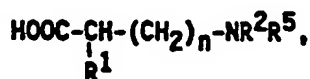
7. Method of preparing compounds having the general formula I characterized by

a) estrifying estramustine with an acid having a reactive substituent giving a compound having a formula II:



wherein R^4 is a reactive substituent and then reacting compound II with an amine having the general formula HNR^2R^3 , or

b) estrifying estramustine with an amino acid having the general formula



wherein R^5 is R^3 or optionally a protecting group, giving an intermediate having the general formula II, wherein R^4 is $-NR^2R^5$, from which the protecting group is afterwards removed for the preparation of compounds having the general formula I.

8. Method according to claim 7 characterized in that compounds wherein n is 0 are prepared.

9. Method according to any of the claims 7-8 characterized in that compounds wherein R^1 is hydrogen are prepared.

10. Method according to any of the claims 7-8 characterized in that compounds wherein R^2 and R^3 are equal or different and are selected from the group consisting of hydrogen, methyl or ethyl.

11. Pharmaceutical compositions containing as an active ingredient one or more of the compounds having the general formula (I), preferably together with a pharmaceutically acceptable carrier and, if desired, other pharmacologically active agents.



European Patent
Office

EUROPEAN SEARCH REPORT

Application number
EP 89111062.9

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
Y	EP, A, 0 104 746 (THE UPJOHN COMPANY) 4 April 1984 * whole document * ---	1-11	C 07 J 41/00, 43/00 A 61 K 31/565, A 61 K 31/58// C 07 C 101/18
Y	US, A, 3 299 104 (HANS JAKOB FEX ET AL.) 17 January 1967 * see claims 1 and 4 * ---	1-11	
Y	GB, A, 962 797 (FARBWERKE HOECHST AG) 1 July 1964 * whole document * ---	1-11	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 07 J
The present search report has been drawn up for all claims			
Place of search STOCKHOLM		Date of completion of the search 04-10-1989	Examiner BERLIN I.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	